

# Rhodora

JOURNAL OF

THE NEW ENGLAND BOTANICAL CLUB

---

Vol. 45.

November, 1943.

No. 539.

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## MICROCHEMICAL STUDIES ON THE GENUS CLADONIA, SUBGENUS CLADINA<sup>1</sup>

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The species of *Cladonia* are distinguished from one another not only by morphological differences but also in many cases by differences in the lichen-acids and related substances which they produce. In the subgenus *Cladina*, for example, it has long been known that *C. rangiferina* (L.) Web. produces atronorine and fumarprotocetraric acid but that *C. sylvatica* (L.) Hoffm., although agreeing with *C. rangiferina* in forming fumarprotocetraric acid, differs in producing usnic acid instead of atronorine. Each of the other species of *Cladina* produces one or two of the three substances mentioned but no species, according to our present information, produces all three.

Prior to the introduction of Asahina's microchemical methods (see Evans, 13) several additional lichen-substances had been found in the *Cladinae* by Zopf and by Hesse (see Sandstede, 14) and some of these had been given names, such as cornicularine, erinacine, laxiuscine, and sylvatic acid. Unfortunately these substances were not fully characterized, and later writers have failed to identify them with any degree of certainty.

One of the first to apply Asahina's methods to a species of *Cladina* was Duvigneaud (5), who examined Belgian specimens of *C. impexa* Harm. He extracted his material with chloroform and treated the residue left after evaporation with Asahina's

<sup>1</sup> Contribution from the Osborn Botanical Laboratory. The writer wishes to thank Professor Alexander Petrunkevitch for the photographs used in Figs. 1, 2 and 6, and Dr. Robert T. Brumfield for the photograph used in Fig. 4.



G. E. solution.<sup>1</sup> In his preparations he found not only the characteristic crystals of usnic acid but also those of another substance, which he described as arborescences formed by aggregation of colorless curved needles. He expressed the opinion that this substance might be the same as erinacine, which was obtained from a form of *C. impexa*, but left the matter in doubt. Duvigneaud obtained identical crystals, unaccompanied by usnic acid, from another Belgian *Cladonia* which was morphologically like *C. impexa*. He considered the absence of usnic acid a sufficient reason for separating this plant from *C. impexa* and described it as a new species under the name *Cladonia subimpexa*.

The results of Duvigneaud's studies were published in 1939, and later in the same year des Abbayes' monograph of the subgenus *Cladina* (4) made its appearance. In this important work the author utilized Asahina's methods for the demonstration of atronorine and usnic acid in the *Cladoniae* and emphasized the value of paraphenylenediamine, usually designated by the letter P, for the detection of fumarprotocetraric acid. In discussing *C. subimpexa*, des Abbayes showed that the production of usnic acid is increased in strong sunlight but that species normally forming this substance may contain only a small amount or even none at all if growing in the shade (4, p. 17). He therefore interpreted *C. subimpexa* as a shade-form of *C. impexa* and reduced it to synonymy under f. *exalbescens* (Vainio) des Abbayes (4, p. 79).

In 1940 Asahina (3) applied his methods to the following representatives of the subgenus: *C. impexa*, *C. pseudoevansi* Asahina (a species proposed as new), *C. Evansi* des Abbayes (see 4, p. 71), and *C. alpestris* (L.) Rabenh. In the first three he was able to demonstrate the presence of perlatolic acid, a substance originally extracted from *Parmelia cetrarioides* Del. var. *typica* DR. (see Asahina 2, p. 40). This substance in the G. E. solution forms characteristic crystals, which are perhaps the same as those found by Duvigneaud in *C. impexa*. Asahina, however, does not suggest that perlatolic acid is identical with any of the substances previously reported in the *Cladoniae*. According to his results the acid is associated with usnic acid in *C. impexa* and *C. pseudoevansi* and with atronorine in *C. Evansi*.

<sup>1</sup> Composed of one part of glycerine and three parts of glacial acetic acid.



Asahina's study of *C. alpestris*, another species producing usnic acid, was based on two series of specimens which differ in their reaction to P. In one series this reagent gives rise to a distinct yellow color; in the other it produces no color change whatever. Plants showing the first reaction had already been observed by des Abbayes, who had separated them from the rest of the species as f. *aberrans* (4, p. 93). He suggested that the substance causing the yellow color might be psoromic acid, and Asahina by means of his microchemical methods was able to prove that this was the case. According to our present knowledge f. *aberrans* is the dominant form of *C. alpestris* in Japan. It occurs also as a rarity in North America but is unknown in Europe.

Asahina's material of *C. alpestris* showing the P— reaction consisted of 15 specimens from Europe, 3 from North America, and 3 from Japan. The specimens from Europe and North America were selected from Sandstede's *Cladoniae exsiccatae*. His preparations were obtained by extracting fragments of the podetia with acetone and by adding the G. E. solution to the dried residue. After the application of gentle heat two types of crystals, which he described as colorless, made their appearance. Since the chemical nature of the substances forming the crystals was unknown to him he designated them by the letters A and B, respectively. According to his brief descriptions crystals of type A, represented by his fig. 8, look like snow-crystals, whereas those of type B, represented by his fig. 9, are in the form of needles aggregated into radiate or tail-like clusters. He compared the latter with the crystals of psoromic and perlatolic acids but stated that they differed from those of psoromic acid in their P— reaction. Unfortunately he neglected to point out any distinctions between crystals of type B and those of perlatolic acid, and the examples shown in fig. 9 are hardly distinguishable from the crystals of perlatolic acid shown in his figs. 1, 5 and 6.

With the exception of *C. pseudoevansi*, which is known only from Japan, the writer has re-examined the species of *Cladonia* studied by Asahina and has been able to confirm his statements regarding *C. impexa*, *C. Evansi*, and *C. alpestris* f. *aberrans*. In the case of P— specimens of *C. alpestris*, however, four different kinds of crystals, instead of two, made their appearance in the G. E. solution, in addition to the crystals of usnic acid. These



crystals were obtained from the numbers of Sandstede's *Cladonias exsiccatae* cited by Asahina, supplemented by other specimens from Europe and North America. In some cases the best precipitation occurred after the preparations had been left standing for several hours, although certain crystals could be demonstrated in the course of a few minutes. Of the four types of crystals found one is clearly the same as Asahina's type A, although it is not wholly without color. The second type, which occurs in over three-fourths of the specimens tested, is referred with considerable hesitation to Asahina's type B. This, too, shows a distinct coloration and differs in several respects from his description and figure. The third type, which is really colorless, is apparently distinct from any of the crystals heretofore found in the *Cladoniae* and may be designated by the letter C. The fourth type, also colorless, is identified with perlatolic acid, since it agrees essentially with the crystals of this substance obtained from *C. impexa* and *C. Evansi*. It is possible, however, that this fourth type may be the same as Asahina's type B.

The application of Asahina's methods to other species of the subgenus *Cladina* soon made it evident that crystals of types A, B, and C are not confined to *C. alpestris*. In *C. mitis* Sandst., for example, according to its present delimitation, all three types occur, type A is found also in *C. sylvatica* (L.) Hoffm. and *C. tenuis* (Floerke) Harm., and type B may be associated with perlatolic acid in *C. impexa*. Attention may be called also to the colorless crystals of another substance which appeared in certain preparations of *C. mitis*. These crystals, which may be tentatively designated by the letter D, resemble the crystals of diffractic acid, as figured by Asahina (1, f. 31), but are apparently not identical with them.

The lichen-substances now definitely known in the *Cladinae* are the following: atronorine, fumarprotocetraric acid, perlatolic acid, psoromic acid, usnic acid, and the substances forming crystals of the types A, B, C, and D. To these may be added rangiformic acid, which has been reported in *C. mitis* by Asahina (see Sandstede, 15, p. 91), and further investigations will undoubtedly bring still other substances to light. Asahina's methods for the demonstration of the first five substances listed have already been summarized by the writer in another connec-



tion (13), but the following descriptions of crystals A, B, C, and D, together with the additional data on the crystals of perlatolic acid, may be of interest to the student. It is hoped that these descriptions, although incomplete, may enable the taxonomist to recognize the various substances under consideration.

#### CRYSTALS OF TYPE A

The crystals, which are here referred to type A, have a yellowish brown color, varying considerably in shade. They apparently

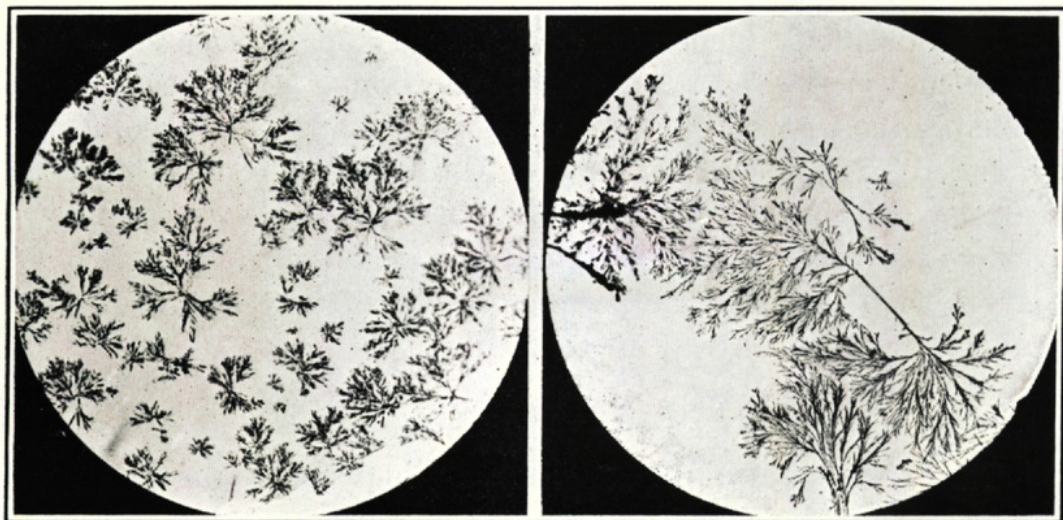


FIG. 1

FIG. 2

FIG. 1. Crystals of type A,  $\times 42$ . Obtained from a specimen of *C. alpestris*, collected in 1933 by Torrey on North Mountain, Green County, New York, No. C-50.

FIG. 2. Crystals of type B,  $\times 20$ . Obtained from a specimen of *C. alpestris*, collected in 1926 by Federov near Twer, Middle Russia, and distributed in Sandstede's *Cladoniae* exsic., No. 1714.

always occur in clusters. These, in their more typical form (Fig. 1) are radiate and show a superficial resemblance to snow-crystals but the rays, which may be simple or variously subdivided, are irregular and vary in number from five to ten. The width of the largest clusters observed was about  $100\mu$ , but it is rare for the width to exceed  $60\mu$ . From the larger clusters, which are similar to those figured by Asahina (3, f. 8), there are all gradations in size down to the smallest clusters, which are barely  $5\mu$  in diameter. In the smaller clusters, the rays are shorter, blunter, and less distinct than in the larger clusters and may not



be present at all. Under such circumstances the clusters form spherical or ellipsoidal masses. The crystals are so closely compacted in the clusters that it is difficult to determine their actual shape. They appear, however, to be in the form of needles or short rods, and in some cases an individual crystal projects from a cluster as a short pointed prism or flat plate 4–6  $\mu$  in width.

The formation of the crystals of type A is in many cases preceded by the appearance of yellowish areas in the preparations. These are mostly confined to the periphery, just within the edge of the cover-glass. The areas contain a profusion of minute oil-like droplets, suspended in a liquid, and it is possible that these may play a part in crystal-formation. As the crystals are precipitated in the yellowish areas the clusters may become surrounded by clear zones or halos. Many of the clusters, however, are not associated with the yellowish areas but are precipitated nearer the center of the preparation. Such clusters tend to be larger than those at the periphery and to show a more typical radiate arrangement.

#### CRYSTALS OF TYPE B

The crystals of type B, according to the writer's interpretation, likewise show a yellowish or yellowish brown color but of a paler shade than in type A. Here, too, the crystals are united into clusters and these in their more characteristic development are in the form of complex branch-systems (Fig. 2), spreading out from a more or less definite point. In typical cases the main axes have a width of only about 1  $\mu$  and are scarcely if at all wider than the ultimate branchlets. Some of the largest and most elaborate branch-systems seen were over 2 mm. long, but these are exceptional and the smallest and simplest clusters are barely 30  $\mu$  long. The branching in general might be described as repeatedly pinnate, although examples of dichotomy are not infrequent. In the larger clusters branching takes place at intervals of 2–15  $\mu$ .

Although in normal examples the ultimate branchlets of a cluster taper to distinct points, there are cases in which a branchlet of this character is connected with a long yellow prismatic crystal having a width of 5–15  $\mu$ . Such a crystal broadens out gradually from a narrow base and then tapers more abruptly to



a point. In all probability it represents a crystal of usnic acid with which a crystal of the B type has coalesced. This hypothesis is supported by the fact that clusters of the B type may be attached by their bases to crystals of usnic acid, as shown by two of the large clusters in Fig. 2, both of which are attached to the same crystal at opposite ends. In combinations of usnic acid with clusters of the B type, the crystals of the latter are usually wider and show a more distinct yellow color than in normal clusters. The greater width, which may be as much as 5–6  $\mu$ , is found not only in the basal portion of a branch-system but also in branches of various ranks, including even the ultimate branches in clusters that are relatively simple. Apparently crystals of the B type are modified in some way by association with crystals of usnic acid, but it is difficult to determine how this is brought about. Since, however, the substance B is known only in species which produce usnic acid, the conditions for associations of this character seem to be always present.

The formation of crystals of type B may be preceded by the appearance of yellowish areas at the periphery of a preparation, similar to those described under type A. In these areas it is not unusual for a branch-system to extend inward from the edge of the cover-glass, and the clusters may be confined to this position, particularly if only a small amount of substance B is present. It may be noted also that crystals of usnic acid extending toward or into the yellowish areas are especially likely to become attached to clusters of type B. The individual crystals of a cluster are in the form of needles but it is difficult to tell where one crystal ends and another begins.

#### CRYSTALS OF TYPE C

The crystals of type C are usually united in clusters but in rare instances isolated crystals can be demonstrated. These are in the form of thin navicular lamellae, pointed at each end and in some cases showing a slight sigmoid curvature (Fig. 3, a). Although the crystals may measure as much as 40–50  $\mu$  in length by 10–12  $\mu$  in width, most of them are considerably smaller. On account of its extreme transparency an isolated crystal is easily overlooked, particularly if it turns one of its faces toward the spectator. The clusters, fortunately, are more conspicuous and



stand out as grayish masses in a field dominated by the yellow crystals of usnic acid. In their simplest arrangement two to many crystals unite by their apices and form subcircular groups about  $15\ \mu$  in diameter. The crystals under these circumstances show their edges and appear as slender rods or needles, in most cases variously curved or distorted. This indicates that they are not necessarily plane lamellae. If the curvatures are in one



FIG. 3

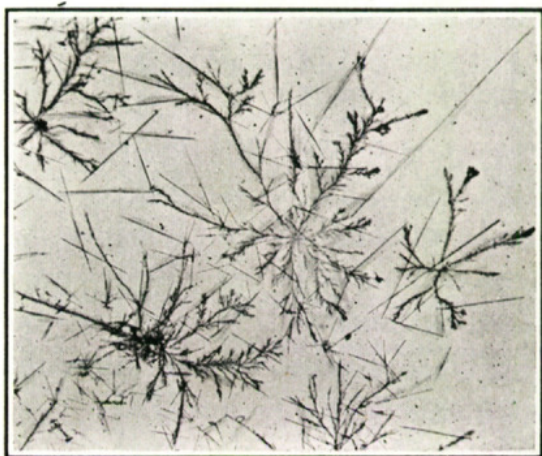


FIG. 4

FIG. 3. Crystals of type C,  $\times 200$ . Obtained from a specimen of *C. submitis*, collected by the writer in 1932 at Killingworth, Connecticut, No. 3120.

FIG. 4. Crystals of type C and of usnic acid,  $\times 28$ . Obtained from a specimen of *C. submitis* f. *prolifera*, collected by the writer in 1941 at Brooklyn, Connecticut, No. 5181a.

direction the cluster exhibits the characteristic appearance shown in Fig. 3, b. This regularity, however, is far from constant and the crystals, particularly in the more crowded clusters, may be curved in various directions (Fig. 3, c, d, e). Clusters in which many or all of the crystals appear straight are likewise not infrequent (Fig. 3, f).

In marked contrast to the clusters showing a subcircular outline are the much larger, irregularly radiate clusters (Fig. 4), which are occasionally produced. These bear a certain resemblance to the aggregates formed by the crystals of types A and B, but are at once distinguished by their total lack of color. The



radiate clusters vary greatly in size and the largest measured had a diameter of 0.25–0.3 mm. The rays are unequal in length and may be simple or variously subdivided. The axis of a ray, which has a width of about 6  $\mu$ , apparently consists of a row of overlapping and coalescent crystals, and upon this axis, throughout the greater part of its length, smaller and variously curved crystals are deposited. These may be scattered but tend to be crowded toward the extremities of the rays.

In some cases a third type of cluster appears at the periphery of a preparation, just within the edge of the cover-glass. The axis here consists of curved crystals seen on edge and attached end to end in such a way that an irregular branch-system is formed. Toward the interior of the preparation some of the branches may gradually broaden and become covered over with smaller crystals, thus acquiring the features of the rays of the radiate clusters.

#### CRYSTALS OF TYPE D

In type D some of the crystals are in the form of exceedingly thin lamellae, others in the form of needles, and between these two extremes are numerous intergradations. In most cases the crystals are combined in clusters, and these may be large enough to extend completely across the field of the microscope. Even the largest clusters are easily overlooked on account of their extreme transparency, and the smaller clusters, which occur far more frequently, are still more inconspicuous. In some of the preparations small groups of lamellate crystals or even individual crystals can be demonstrated (Fig. 5, a). These are bounded by straight lines and are longer than broad with parallel sides. The ends may be bounded by two lines meeting at an obtuse angle or by continuous lines meeting the sides squarely or obliquely. Compound crystals are occasionally met with (Fig. 5, b). In the examples studied the lamellae varied in width from 2  $\mu$  to 50  $\mu$  and one of the largest seen had a length of 0.2 mm. In many instances the process of crystallization is incomplete and the bounding lines are zig-zag or otherwise irregular.

When the lamellae are seen on edge they appear as thin lines, which may be straight but which more frequently are curved in the form of a "C", showing that the crystals may be concave.



In clusters composed of lamellae alone the crystals may be superimposed upon one another (Fig. 5, c) or some of the crystals may be attached by their edges to the faces of other crystals. The latter condition is shown in Fig. 5, d, which represents two clusters composed of both lamellate and acicular crystals. It will be noted that the needles are attached to the lamellae at irregular intervals and that they form simple tufts or more elaborate branch-systems. In Fig. 5, e, a cluster composed entirely of somewhat shorter needles is shown. The cluster is in the form of a more or less pinnate branch-system, and even more complicated clusters of a similar type are not unusual. The needles of type D are in some cases so fine and delicate that they almost escape observation. A dense tuft of these exceedingly fine needles may show a pale brownish shade, although the majority of the clusters appear absolutely colorless.

In the subgenus *Cladina* crystals of the D type seem to be restricted to *C. mitis*. In the subgenus *Cenomyce*, however, the writer has demonstrated their presence in certain specimens of *C. rangiformis* Hoffm., a close relative of the common and widely distributed *C. furcata* (Huds.) Schrad. The crystals were found, for example, in Nos. 350, 490, 563, 684, 686, and 688 of Sandstede's *Cladoniae exsiccatae*, coming from various localities in northern and central Europe, although they were lacking in No. 173 from Norway and in No. 546 from Brandenburg. These data are sufficient to show that the substance D may be regarded as an accessory component of *C. rangiformis*. According to Zopf (16, p. 89) rangiformic acid also is a substance which may or may not be present in *C. rangiformis*, and Asahina (as already noted) has demonstrated the presence of this acid in *C. mitis*. It would of course be premature to suggest that there is any connection between the substance D and rangiformic acid, and yet it would not be surprising if further investigations proved them to be identical. Unfortunately, according to Asahina, rangiformic acid can not be demonstrated by microchemical methods.

#### CRYSTALS OF PERLATOLIC ACID

The crystals which perlatolic acid forms in the G. E. solution have been described by Asahina (2, p. 40). According to his account they are colorless and in the form of needles arranged in



bundles, which are grouped in irregular radiate clusters. These are clearly represented in his original figure (2, pl. 1, f. 3), which was based on preparations obtained from *Parmelia cetrarioides* var. *typica*. They are equally well shown in preparations obtained from *Cladonia impexa*, *C. pseudoevansi*, and *C. Evansi* (3, f. 1, 5 and 6). In the writer's experience the radiate clusters are usually precipitated at some distance from the edge of the cover-glass. The crystals of which they are composed are in the form

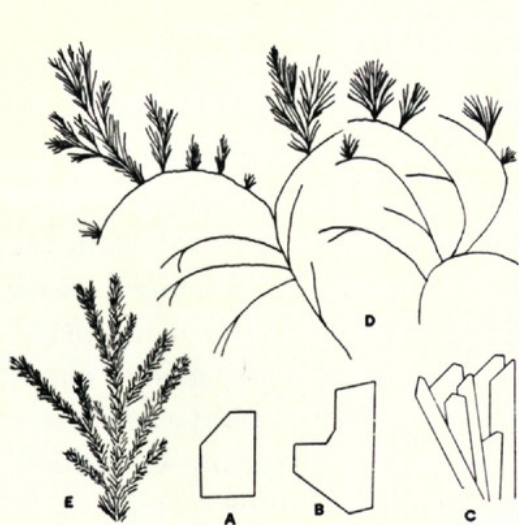


FIG. 5



FIG. 6

FIG. 5. Crystals of type D,  $\times 200$ . Obtained from a specimen of *C. mitis*, collected in 1941 by Luttrell on Bald Knob, Giles County, Virginia, No. 2683.

FIG. 6. Crystals of perlatolic acid,  $\times 20$ . Obtained from a specimen of *C. alpestris*, collected in 1941 by Sheldon near Siasconset, Nantucket, Massachusetts, No. S-145.

of long straight needles or narrow prisms, varying in width from less than  $1\ \mu$  to  $20\ \mu$ . Many of the crystals are simple throughout their entire length but others branch, usually by forking, and this process may be repeated. In most of the clusters one or more of the broader prisms, which taper to slender points, become covered, especially toward their tips, with loose or dense masses of short secondary crystals in the form of straight needles.

At the periphery of a preparation a somewhat different type of crystal-aggregate may make its appearance. This consists of one or more axial crystals which extend inward at right angles or obliquely from the edge of the cover-glass (Fig. 6). An axial



crystal, which is prismatic in form, broadens out gradually from a narrow base to a width of 6–8  $\mu$  and then tapers more abruptly to a sharp point. Upon this crystal toward its inner end a loose or dense mass of short acicular crystals is deposited, similar to those associated with the broader prisms of the radiate clusters. Unless these secondary crystals are sparingly produced the tip of an axis becomes completely concealed. Toward the base of the axial crystals slender branches may be given off, in some cases very abundantly. These in turn may branch repeatedly and form loose and more or less interwoven branch-systems.

#### DISTRIBUTION OF LICHEN-SUBSTANCES IN *C. ALPESTRIS*

Although six different lichen-substances are now known in *C. alpestris*, usnic acid alone seems to be constant. In some specimens, indeed, this is the only lichen-substance that can be demonstrated, but such specimens are unusual. In most cases one or more of the other substances accompany the usnic acid, and if two or three are present these may occur in various combinations. In order to learn something definite about the distribution of lichen-substances in the species the writer has examined a series of specimens from Europe and a second series from North America.

The European specimens, all giving the P — reaction, include the following numbers from Sandstede's *Cladoniae exsiccatae*, under each of which the substances demonstrated are indicated by the letters A, B, and C, for the crystals designated by these letters, and by the abbreviation "Per.", for the crystals of perlatolic acid: 85 (B), 86 (B, Per.), 110 (A), 565 (A, B), 823 (A, B), 877 (B), 1020 (B), 1146 (A, B), 1232 (B), 1462 (B), 1463 (A, B), 1680 (B, C), 1701 (A, B), 1714 (B, Per.), 1715 (A, B, C, Per.), 1782 (A, B, Per.), 1797 (A, B, Per.), 1810 (A, B, C), and 1811 (A, B). These specimens came from various localities in central and northern Europe, and all but four (Nos. 85, 86, 1680, and 1715) are cited by Asahina (3, p. 190). No. 1715, which contains all four substances, was collected by Mrs. E. J. Dostojnova in northern Russia. Six additional European specimens were likewise examined, as follows: Utviken, Norway, *Sandstede* (B); Stegafyeld, Norway, *Sandstede* (B, C); Svalestein, Norway, *Havaas*, distributed in Lich. Norvegiae Occid. exsic., No. 76 (B);



Delarna, Sweden, *Holdsworth*, No. 6 (usnic acid only) and No. 7 (B); Tirol, *Arnold*, distributed as f. *inturgescens* Arnold in Lich. exsic., No. 1020 (A); and Bohemia, *Anders* (B, Per.).

These data show that substance A is present in 12 of the 27 specimens examined (about 46 per cent), B in 23 (about 85 per cent), C in 4 (about 15 per cent), and perlatolic acid in 6 (about 22 per cent). They show further that A and B accompany each other in 10 specimens (about 38 per cent) and that C and perlatolic acid are both accompanied by B. The figures given must not be accepted too literally. In some cases two or even three tests were required before characteristic crystals appeared, and it is therefore possible that some of the figures may be too low. At the same time they give a general idea of the distribution of substances A, B and C and of perlatolic acid in European material of *C. alpestris*.

The North American material of *C. alpestris* examined by the writer includes five specimens which give a yellow color with P and which therefore represent the following form:

CLADONIA ALPESTRIS f. ABERRANS des Abbayes, Bull. Soc. Sci. Bretagne **16** (fasc. 2): 93. 1939. In the citation of the specimens the lichen-substances present are indicated and the abbreviation "Ps." designates psoromic acid. ALASKA: White House Pass, *Heath*, 1903 (A, B, Per.). QUEBEC: Tadousac, *Torrey*, 1937 (B, Per., Ps.); Mt. Richardson, *Torrey*, 1937 (A, B, Per., Ps.). PENNSYLVANIA: Lake Shehawken, *Dix*, 1939 (A, B, Per., Ps.).<sup>1</sup> MICHIGAN: Carp Lake, Porcupine Mountains, Ontonagon County, *Nichols*, 1935 (A, Per., Ps.). Des Abbayes lists f. *aberrans* from Canada and from the island of St. Paul in the Bering Sea, as well as from Japan.

His record for Canada was based on *Torrey's* specimens from Quebec. It will be seen that psoromic acid has been demonstrated in four of the specimens listed. Repeated tests, however, gave negative results with the specimen from Alaska. This may have been due to the difficulty, which *Asahina* emphasizes, of detecting psoromic acid in the presence of an excess of usnic acid.

The North American material of *C. alpestris*, in which P produces no change in color, totals 119 specimens. These represent a distribution extending from Alaska and Labrador southward to Minnesota, Wisconsin, Michigan, and West Virginia. Seven of

<sup>1</sup> Reported as *C. alpestris* by *Dix* in *Torrey* **40**: 45. 1940.



the specimens were distributed in exsiccati, as follows:—Macoun, Lichenes exsic., No. 55, "common in Gaspé and on Anticosti, and in the Rocky and Selkirk Mountains; also north shore of Lake Superior, 1869," distributed as *C. rangiferina* var. *alpestris* (B). Cummings and Seymour, Decades N. Am. Lichens, No. 32, Wellesley, Massachusetts, 1891, distributed as *C. rangiferina* c. *alpestris* (A, B, Per.). Merrill, Lich. exsic., No. 6, Rockport, Maine, 1908 (A, B); No. 57, same locality, 1925 (usnic acid only). Sandstede, *Cladoniae* exsic., No. 667, Minnesota, Fink, 1894 (B); No. 1220, Rockland, Maine, Merrill, 1924 (A); No. 1567, Wareham, Massachusetts, Robbins, 1925 (B). The last three specimens are among those listed by Asahina (3, p. 190). Without enumerating the other specimens the distribution of substances A, B and C and of perlatolic acid may be summarized. Of the 118 specimens examined 56 (or about 47 per cent) were found to contain substance A, 79 (or about 76 per cent) substance B, 10 (or about 8 per cent) substance C, and 33 (or about 28 per cent) perlatolic acid. These numbers do not diverge very widely from the percentages 46, 85, 15, and 22, respectively, derived from European material.

The question now arises, what is the taxonomic significance of the various substances found in *C. alpestris*? In other words should plants producing the substance A, for example, be separated as a distinct variety or form from plants which do not produce this substance? This question might be answered in the affirmative: (1) if plants producing the substance A were also distinguished by morphological differences; (2) if they occupied a restricted geographical range; or (3) if the presence of A excluded B, C, or perlatolic acid, either singly or in combination. According to our present knowledge not one of these conditions is fulfilled. The writer suggests, therefore, that A, B, C and perlatolic acid, so far as *C. alpestris* is concerned, be regarded merely as accessory substances having no taxonomic significance.

Whether an exception should be made in the case of f. *aberrans* is also open to question. This form, to be sure, is characterized by a distinct color-reaction with P, but this depends simply upon the presence of psoromic acid. There are, unfortunately, no morphological features characterizing f. *aberrans*, and the psoromic acid may be accompanied by perlatolic acid, as well as



by the substances A and B. Since, however, f. *aberrans* is known only from North America and Japan, it perhaps deserves recognition on the basis of its restricted geographical distribution.

#### DISTRIBUTION OF LICHEN-SUBSTANCES IN *C. MITIS*

According to the results obtained by the writer usnic acid is the only constant constituent of *C. mitis*, the species agreeing in this respect with *C. alpestris*. The following account of the distribution of the other lichen-substances found in the species is based on 125 specimens from Europe, 524 from North America, and 4 from Chile and New Zealand. The species is known also from Asia and Africa (see des Abbayes 4, p. 127), but the writer has seen no specimens from either of these continents.

The European material examined includes a long series from Sandstede's *Cladoniae exsiccatae*, 6 numbers from des Abbayes' Lichenes Gallici, and about 50 packets from other sources. In a large number of these specimens only usnic acid could be demonstrated. This was true of the following numbers from Sandstede's *Cladoniae exsiccatae*: 55 (the type of the species from Oldenburg), 56, 58, 59, 63, 64, 66, 68, 119, 120, 261, 295, 323, 566, 793, 794, 822, 984, 985, 1033, 1037, 1058, 1060, 1518, 1440, 1441, 1516, 1517, 1761, 1762, 1809, 1816, and 1817. The same result was obtained from 20 other specimens, giving a total of 53, or about 42 per cent of all the specimens examined. The following numbers of Sandstede's *exsiccatae* showed crystals of the A type, unaccompanied by either B or D: 57, 60, 61, 65, 67, 69, 117, 118, 121, 247, 402, 683, 717, 735, 795, 797, 983, 994, 1056, 1057, 1059, 1115, 1439, 1519, 1520, 1521, 1644, 1645, 1646, 1781, 1818, 1819, 1820, and 1869. Adding to these 17 other specimens in which only crystals of the A type could be demonstrated gives a total of 51. Crystals of the B type, accompanied by crystals of the A type were found in only 2 specimens, Nos. 1519 and 1647 of Sandstede's *exsiccatae*, representing less than 2 per cent. Nos. 324, 796, 1306, and 1696 of Sandstede's *exsiccatae*, together with 11 other specimens, yielded crystals of the D type, a total of 15, or about 12 per cent. In three cases both A and D were present. The number of specimens, therefore, in which crystals of the A type were demonstrated totals 56, or about 45 per cent.

Des Abbayes (4, p. 121) emphasizes the fact that an occasional



specimen of *C. mitis*, a typically P— species, turns yellow or pale reddish, particularly at the tips of the podetia, in the presence of this reagent. Although he mentions no definite specimens that give a yellow color, he lists the following numbers from Sandstede's exsiccatae in which he obtained a pale reddish reaction: 323, 792, 793, 994, 1056, 1603, 1748, 1750, 1761, 1820, 1860, and 1869. Seven of these are included in the lists given above and three are lacking in the writer's set of the exsiccatae, but Nos. 1603, 1860, and 1869 deserve especial mention. In No. 1603 a definite red color appeared upon the addition of P, indicating that this number (at least in part) represents *C. tenuis*, instead of *C. mitis*. No. 1860, on the other hand, gave a distinct yellow color in the presence of P and yielded characteristic crystals of psoromic acid when tested by Asahina's methods. This number was collected near Upsala, Sweden, by Hedlund in 1928. In No. 1869, which gave a similar yellow color, no crystals of psoromic acid could be demonstrated. Whether or not specimens of *C. mitis*, which give a yellow color with P, should be separated as a distinct form, comparable with *C. alpestris* f. *aberrans*, may be left for European students to decide. No specimens of this type have as yet been detected in North America.

The following numbers from des Abbayes' Lichenes Gallici showed the presence of perlatolic acid accompanied by substance B: 41 (distributed as f. *prostrata* Sandst.), 42 (distributed as f. *vesciculosa* Sandst.), 43 (distributed as f. *prostrata ad vesciculosam*) and 44 (distributed as f. *divaricata* Sandst. *ad vesciculosam*). These specimens were collected by des Abbayes in 1937 at le Pouliguen, Loire-Inférieure, France, where they grew closely intermingled. In commenting upon them Sandstede (15, p. 14) states that No. 42 bears a strong resemblance to "*Cl. impexa-spumosa*" and "*Cl. sylvatica-subpumosa*," implying that it is not distinguished by definite morphological features. Since perlatolic acid has not been found in any other material referred to *C. mitis* and since it represents a characteristic constituent of *C. impexa*, the writer suggests that these four numbers be transferred to *C. impexa*. The P— reaction would of course distinguish them from *C. sylvatica*, which is definitely P+.

The North American specimens in the Yale Herbarium, which have been referred to *C. mitis* are definitely P—. They fall



naturally into two groups, which differ from each other in the lichen-substances produced, in geographical distribution, and in certain morphological features. Specimens belonging in the first group do not differ materially from European material either chemically or morphologically. Some, for example, contain usnic acid only, whereas others yield in addition crystals of the A, B, or D type, either singly or in certain combinations. The material of this group exhibits a geographical distribution extending from Alaska and Arctic America southward through Canada, northern New England and northern New York to Oregon, Wyoming, Wisconsin, and Virginia.

Specimens belonging in the second group yield, in addition to usnic acid, crystals of the C type. These in some cases are accompanied by crystals of the A type but apparently never by those of either the B or D type. The range of the second group extends from the Cape Cod region southward, mostly near the coast, to Delaware, Virginia, and West Virginia. It is therefore much more restricted than that of the first group, and it may be noted also that specimens of either group rarely occur within the range of the other. The morphological features of the second group will be considered later.

The material of the first group, 186 specimens in all, includes the following specimens from exsiccati:—Merrill, Lich. exsic., No. 104, Rockland, Maine, 1909, distributed as *C. sylvatica* f. *morbida* (A). Sandstede, *Cladoniae* exsic. No. 1223, Rockland, Maine, Merrill, 1924 (A); No. 1469, Brandon, Vermont, Dutton, without date (A); No. 1476, Wareham, Massachusetts, Robbins, 1925 (A); No. 1749, Bourne, Massachusetts, Robbins, 1927 (usnic acid only); No. 1566, Jackson, New Hampshire, Robbins, 1924 (A, D); No. 1582, same station and collector, 1925 (A, D); and No. 1833, same station and collector, 1927 (usnic acid only). The enumeration of the other specimens may be omitted, as in the case of the North American material of *C. alpestris*, but the distribution of substances A, B and D may be summarized. Of the 186 specimens examined 125 (or about 67 per cent) showed crystals of type A, 9 (or about 5 per cent) crystals of type B, and 24 (or about 13 per cent) crystals of type D. The corresponding percentages for the European material were 45, 2, and 12. The marked discrepancy in the case of substance A is due to the fact



that the European material includes relatively more specimens containing usnic acid only, possibly because many of these specimens came from a restricted area.

The specimens examined from the Southern Hemisphere are the following:—CHILE: Port Marqueri, *Junge*, 1932 (A, B). NEW ZEALAND: near Atiamuri, Allison, 1931 (usnic acid only); same station and collector, 1932, two packets (B).

In estimating the taxonomic significance of the various lichen-substances in *C. alpestris* three criteria were suggested. If the same criteria are employed in the case of *C. mitis*, it might appear that plants containing substance B or substance D had certain claims for recognition as distinct forms. Although each of these substances may accompany A, they are apparently never found together. Plants containing B, moreover, are mostly northern or antarctic and plants containing D are in many cases smaller and more delicate than plants in which this substance is lacking. The substance B, in fact, is often present in specimens representing the northern and antarctic f. *laevigata* (Vainio) des Abbayes (4, p. 122), in which the pseudocortex is poorly developed; and the substance D in specimens representing f. *tenuis* Sandst. (14, p. 110). It would therefore be a happy solution of the problem if B could be definitely associated with f. *laevigata* and D with f. *tenuis*. There are reasons, unfortunately, why this can not be done. Certain plants containing B, for example, agree neither in range nor in their morphological features with f. *laevigata*, and certain plants containing D are too robust to be referred to f. *tenuis*. It thus seems advisable, under the circumstance, to regard substances B and D, as well as substance A, as accessory constituents of *C. mitis* and to assign to them no taxonomic importance.

The case is different with specimens containing the substance C. Although the morphological distinctions between these specimens and those belonging to the first group are slight and less definite than might be desired they are sufficient in the case of well-developed material for the separation of the groups. According to des Abbayes (4, p. 120) the sterile ultimate branchlets of the podetia in *C. mitis* are mostly in 2's or 3's, relatively long, slender, and (in most cases) curved. These features are more or less in evidence in European material and in North



American material belonging to the first group. In material containing the substance C, however, the ultimate branchlets present a different appearance. They are not only more numerous and shorter than in the first group but the curvature tends to be less marked. Many of the branchlets, in fact, are not curved at all. The podetia, moreover, tend to be more robust than in the first group. These slight morphological differences, the restricted geographical distribution of the second group, and the fact that the presence of C apparently excludes B and D afford a sufficient basis for the separation of the group from *C. mitis* as a distinct species, as follows:—

*CLADONIA submitis*, sp. nov. Thallus primarius ignotus. Podetia typice straminea vel albido-straminea, sat robusta, caespitoso-conferta, longitudine vulgo 5–7 cm., crassitudine vulgo 1–3 mm., ad axillis saepe dilatata, parce dichotome, abundantius polychotome ramosa, base divisionibus inaequalibus axem sympodialefficientia, ramis et ramulis ramosis, ramulis superioribus abbreviatis, rectis vel rarius nutantibus, impellucida, glabra vel subverruculosa, K—, P—, acidum usnicum et materiam "C" continentia.

Although *C. submitis* is not confined to sandy localities it shows a strong preference for areas in which sand is abundant. Under favorable conditions the podetia form compact and extensive colonies, in which the more centrally situated individuals are erect or ascending. In many cases, however, the podetia are prostrate or nearly so and are either scattered about on the substratum or form loose clusters. In some cases tufts of the species grow in mats of *Polytrichum*.

The material of *C. submitis* examined by the writer includes 338 specimens and in 80 of these, representing about 24 per cent, crystals of the A type are associated with crystals of the C type in the G. E. preparations. No. 1391 of Sandstede's *Cladoniae exsiccatae*, collected by Robbins at Wareham, Massachusetts, in 1924, may be designated the type of the species. This number was distributed as *C. mitis* m. *prostrata* Sandst. Other stations for the species are as follows:—

VERMONT: Brattleboro, *Evans*, 1935; Halifax, *Evans*, 1939; Marlboro, *Evans*, 1939; and Putney, *S. M. Thomson*, 1942. These specimens are less robust than most of the material from farther south. MASSACHUSETTS: Brewster, *Evans*, 1929; Provincetown, *Evans*, 1930; Stoughton, *Blake*, 1931; Manchester,



*Harris and Smith*, 1934; Eastham, *Evans*, 1935, and *Torrey*, 1936; Wellfleet, *Evans*, 1935, *Torrey*, 1937, and *Logan*, 1937; Martha's Vineyard, *Torrey*, 1936, Craigsville Beach, *Torrey*, 1936; Cape Race, *Torrey*, 1936; South Chatham, *Torrey*, 1936; Nanset Coast Guard Station, *Torrey*, 1936; Nantucket, *Torrey*, 1937, and *Sheldon*, 1940; Centerville, *Torrey*, 1937; Clinton, *Harris et al.*, 1938; and Tuckernuck, *Sheldon*, 1938. Many of these stations in the Cape Cod region. RHODE ISLAND: South Kingstown, *Evans*, 1929. CONNECTICUT: stations previously listed by the writer under *C. mitis* (6, p. 381, 7, p. 123, 8, p. 36, and 9, p. 8). NEW YORK: Fisher's Island, *Evans*, 1930; numerous stations on Long Island, *Torrey*, *Latham*, and *Cain*, 1933-1939; and Croton Aqueduct near Quaker Bridge, *Torrey*, 1937. NEW JERSEY: the stations previously listed by the writer under *C. mitis* (10, p. 86, 11, p. 138, and 12, p. 143). DELAWARE: Cape Henlopen and Reheboth, *Torrey*, 1936. VIRGINIA: Short Mountain, *Allard*, 1938, and *Luttrell*, 1942; and Ironto, *Gray*, 1940, and *Luttrell et al.*, 1941. WEST VIRGINIA: Petersburg, *Gray*, 1939.

The following forms of *C. submitis*, analagous to the similarly named forms of *C. mitis*, may be distinguished:—

CLADONIA SUBMITIS f. **divaricata** f. nova, podetia repitito verticillate ramosa.

No. 1565 of Sandstede's *Cladoniae* exsiccatae, collected by Robbins at Wareham, Massachusetts, in 1925, and distributed as *Cl. mitis* m. *divaricata*, may be designated the type of this form. No. 1750 of the same series, collected by Robbins at the same locality, in 1927, and distributed as *Cl. mitis-divaricata*, likewise belongs here. Other records follow.

MASSACHUSETTS: Wareham, *Robbins*, 1923 and 1924; Brewster, *Evans*, 1929; Centerville, *Torrey*, 1937; and Nantucket, *Sheldon*, 1940. RHODE ISLAND: South Kingstown, *Evans*, 1929; Watch Hill, *Evans*, 1935. CONNECTICUT: various stations previously listed by the writer under *C. mitis* f. *divaricata* (6, p. 383, and 8, p. 36). NEW YORK: Fisher's Island, *Evans*, 1930; and Long Island, *Latham*, 1938. NEW JERSEY: the stations previously listed by the writer under *C. mitis* f. *divaricata* (10, p. 86).

CLADONIA SUBMITIS f. **prolifera** f. nova, podetia vulgo decumbentia, ramulis adventiciis brevibus plus minusve numerosis tecta.

This form may be typified by No. 1564 of Sandstede's *Cladoniae* exsiccatae, collected by Robbins at North Falmouth, Massachusetts, in 1924, and distributed as *Cl. mitis* m. *prolifera*. The following additional stations may likewise be cited:—

MASSACHUSETTS: Wareham, *Robbins*, 1923; Martha's Vineyard, *Harris*, 1934, and *Torrey*, 1936; Cape Race, *Torrey*, 1936; South Chatham, *Torrey*, 1936; and Merrimack, *Harris*, 1938. CONNECTICUT: the stations previously listed by the writer under



*C. mitis* f. *prolifera* (6, p. 383, 7, p. 123, 8, p. 36, and 9, p. 8). NEW YORK: Long Island, *Torrey*, 1933, and *Latham*, 1934. NEW JERSEY: the stations previously listed by the writer under *C. mitis* f. *prolifera* (10, p. 86, 11, p. 138, and 12, p. 143). DELAWARE: Cape Henlopen, *Torrey*, 1936.

CLADONIA SUBMITIS f. **soralifera** f. nova, podetia farinoso-sorediosa, sorediis luteo-albis, in maculis circumscriptis. This form is known at present only from the following station:

CONNECTICUT: North Haven, *Miss Fulford*, 1932, previously listed by the writer (8, p. 37) as *C. mitis* f. *soralifera* Sandst.

#### DISTRIBUTION OF LICHEN-SUBSTANCES IN ALL THE SPECIES OF THE SUBGENUS CLADINA

Although several species of *Cladina* have not yet been studied critically by Asahina's methods a summary of our present knowledge regarding the distribution of the various lichen-substances found in the group may be of interest and is given in Table 1. In addition to the ten species listed by des Abbayes in his monograph (4) the list includes *C. pseudoevansii* and *submitis*. Following the example of its author the North American *C. tenuis* subspecies *subtenuis* des Abbayes is not separated specifically from *C. tenuis*. Of the species listed *C. leucophaea* is apparently confined to Europe and *C. pseudoevansi* to Japan. The remaining ten species have all been reported from North America, and Nos. 1, 4, 6, 8, 11, and 12 are widely distributed in other parts of the world. The sign + indicates a constant component, the sign  $\pm$  an accessory component.

TABLE 1

	Atronorine	Fumarprotocetraric acid	Perlatolic acid	Psoromic acid	Rangiformic acid	Usnic acid	Substance A	Substance B	Substance C	Substance D
1. <i>C. alpestris</i> (L.) Rabenh.			$\pm$	$\pm$		+	$\pm$	$\pm$	$\pm$	
2. <i>C. Evansi</i> des Abbayes	+		+			+	$\pm$	$\pm$	$\pm$	
3. <i>C. fallax</i> des Abbayes		+				+				
4. <i>C. impexa</i> Harm.			+			+		$\pm$		
5. <i>C. leucophaea</i> des Abbayes		+								
6. <i>C. mitis</i> Sandst.				$\pm$	$\pm$	+	$\pm$	$\pm$		$\pm$
7. <i>C. pseudoevansi</i> Asahina			+			+				
8. <i>C. rangiferina</i> (L.) Web.	+	+								
9. <i>C. Sandstedei</i> des Abbayes	+	+								
10. <i>C. submitis</i> Evans						+	$\pm$		+	
11. <i>C. sylvatica</i> (L.) Hoffm.		+				+	$\pm$			
12. <i>C. tenuis</i> (Floerke) Harm.		+				+	$\pm$			



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## CARUNCULATE SEED DISSEMINATION BY ANTS

BURTON N. GATES

THE caruncle-bearing seeds of the Large White Trillium and of the Bloodroot, have been shown to be disseminated by ants.<sup>1</sup> They are attracted by the caruncle, as a lure and a probable source of food. It was observed that the seeds were carried into the ants' nest, the caruncle removed, and the denuded seeds discarded outside of the nest. This the writer reported in RHODORA.

<sup>1</sup> Burton N. Gates, Dissemination by Ants of the Seed of *Trillium grandiflorum*. RHODORA, 1940, vol. 42, p. 194.

*Ibid.* Observations in 1940 on the Dissemination by Ants of the Seeds of *Trillium grandiflorum*. RHODORA, 1941, vol. 43, p. 206.

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